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Brief report
Interleukin-6 gene polymorphism and risk of osteoarthritis of the hip: a case–control study

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Summary

Objectives: Osteoarthritis (OA) is considered a polygenic disease controlled by the expression of genetic factors. Genes encoding for cytokines have been associated with susceptibility for joint OA and interleukin (IL)-6 gene is also supposed to be involved in the cartilage degradation process. In this case–control study, we evaluated for the first time whether the risk of hip OA might be influenced by the −174 IL-6 gene polymorphism.

Methods: The distribution of IL-6 genotypes was evaluated in 75 patients affected by hip OA and in 107 age- and sex-matched controls.

Results: The distribution of IL-6 genotypes in (1) patients with hip OA: 33 GG, 30 GC, 12 CC and (2) control subjects: 34 GG, 40 GC, 33 CC. The frequency of the CC genotype was significantly higher in control patients ($P = 0.02$). Logistic regression analysis indicated that the presence of the CC genotype is independently associated with a decreased risk of OA (odds ratio 0.4 [95% confidence interval 0.1–0.9], $P = 0.04$).

Conclusions: Primary OA of the hip has an important genetic component and variations of genes encoding for inflammatory cytokines, such as IL-6, may play an important role in the series of events responsible for the pathophysiology of OA.

Key words: Hip osteoarthritis, Interleukin-6, Gene polymorphism.

Introduction

The incidence of osteoarthritis (OA) has increased in western countries in the last decades, accordingly with the demographic phenomenon of aging population[1]. Although many risk factors have been associated with OA, including age, previous injury, obesity, diet, hormone therapy, and smoking habits[2,3], the pathogenesis of OA is still incompletely characterized. Cellular and molecular inflammatory mechanisms may be important in the development of OA, as suggested by the accumulation of inflammatory cells and mediators in the site of cartilage damage and degeneration.

Many studies suggest that primary OA of the hip has an important genetic component and OA is considered a polygenic disease controlled by the expression of genetic factors. Also, cytokine genes have a relevant role in regulating the catabolic/anabolic balance of articular cartilage, and recent studies[4,5] have confirmed the association of the interleukin (IL)-1 gene cluster and IL-1 receptor antagonist (IL-1RA) with radiographic signs of OA of the hip. In this setting, a crucial role has been hypothesized for IL-6, which is produced by several cell types and plays a key role in driving the acute inflammatory response. The human IL-6 gene is mapped to chromosome 7p21-24 with an upstream promoter containing 303 bp. A single nucleotide polymorphism (SNP) has been described at position −174 of the promoter region of the IL-6 gene, resulting in three possible genotypes, GG, GC, and CC. This polymorphism has been associated with the prevalence, incidence, and/or prognosis of a variety of diseases, such as Alzheimer's disease, atherosclerosis, cardiovascular disease, cancer, non-insulin-dependent diabetes mellitus, osteoporosis, sepsis, and systemic-onset juvenile chronic arthritis[6].

In this case–control study, we evaluated for the first time whether the risk of hip OA might be influenced by the IL-6 gene polymorphism. Seventy-five patients affected by hip OA and 107 age- and sex-matched controls were studied. The distribution of IL-6 genotypes in (1) patients with hip OA: 33 GG, 30 GC, 12 CC and (2) control subjects: 34 GG, 40 GC, 33 CC. The frequency of the CC genotype was significantly higher in control patients ($P = 0.02$). Logistic regression analysis indicated that the presence of the CC genotype is independently associated with a decreased risk of OA (odds ratio 0.4 [95% confidence interval (CI) 0.1–0.9], $P = 0.04$).

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These results support the hypothesis that variations of genes encoding for inflammatory cytokines, such as IL-6, may play an important role in the series of events responsible for the pathophysiology of OA.

Materials and methods

Patients and controls were recruited among subjects consecutively admitted to the Department of Orthopedics at the A. Gemelli University Hospital of Rome, Italy, from October 2003 to March 2004. All patients underwent anterior–posterior and axillary hip radiographs and antero-posterior hand radiographs using standard procedures. Diagnosis of hip OA was made according to established criteria[7], submitting all patients to plain pelvic radiograph at entry into the study. Antero-posterior radiograph was performed with the patient standing on both legs. The patient's feet were $15° \pm 5°$ internally rotated. The X-ray beam was horizontal, perpendicular to the table. The source to film distance was 100 cm. Radiographs were assessed by one expert reader. For quantitative measurement, the interbone distance at the narrowest point was measured in millimeters using a 0.1 mm graduated magnifying glass laid directly over the radiograph. In addition, joint space narrowing was graded 0–3 using a radiographic atlas[8]. Overall severity of OA was graded using the Kellgren and Lawrence (KL) scale[9]. All patients selected as cases had a minimal joint space $\leq$1.5 mm, their KL score was 3–4, and all of them underwent surgery for total hip arthroplasty. Otherwise, none of the cases had bilateral hip OA and the major number of these patients had superolateral hip OA with an indeterminate pattern. Generalized OA (GOA) was defined by the hypothesis that OA found in the hand is an indicator of disease in other large joints. An individual was considered to have GOA if more than three interphalangeal joints scored at KL grades 2–4 (see Refs.[9,10]). With these criteria, none of the studied individuals had GOA.

Subjects matching the cases for age and sex, without clinical and radiographical evidence of OA, were used as controls. These subjects had no relationship with cases and no family history of OA. Controls were enrolled between subjects who were admitted to the ambulatory of the Department of the Orthopedics for post-traumatic injuries or other symptomatic problems which did not correlate with OA. All control subjects underwent clinical examination and X-ray evaluation, in order to exclude the presence of hip OA.

A total of 100 patients and 150 controls were initially selected. Then, subjects affected by tumors, chronic inflammatory diseases, infective diseases, and autoimmune diseases were excluded. After excluding these cases, a total of 75 patients with hip OA and 107 controls were enrolled.

All subjects were Caucasians from central and southern Italy and belonged to independent pedigrees. For all individuals enrolled in the study, a complete medical history was collected and included smoking habit, hormonal or metabolic disorders, and previous joint injury. Body Mass Index (BMI) was calculated as weight/height^2 ratio. Informed consent was obtained from all patients. The study protocol was accepted by the Ethics Committee of our University Hospital.

Peripheral blood samples were obtained from all subjects and nucleic acid isolation was carried out from 200 μl of peripheral blood by using a DNA extraction kit (NucleoSpin Blood QuickPure, Macherey-Nagel, Duren, Germany), as recommended by the supplier. Genomic DNA was assayed with polymerase chain reaction (PCR) for the detection of IL-6 gene, using the published primer set: 5′-TGACTTC AGCTTTACTCTTTGT-3′ (sense primers) and 5′-CTGATTGGAAACCTTATTAGG-3′ (antisense primers). Briefly, PCR reaction, containing 0.2 mmol/l of each primer, 0.2 mmol/l of Deoxyribonucleoside Triphosphates (dNTPs) (Boehringer GmbH, Mannheim, Germany), 1.5 mmol/l $MgCl_2$, and 2.5 U of AmpliTaq polymerase (Perkin–Elmer, Cetus, Norwalk, CT) in a final volume of 20 μl, was performed in a GeneAmp PCR System 9700 (Perkin–Elmer) with an initial denaturation step of 10 min at 94°C and a final extension step of 10 min at 72°C. The following thermal profile was repeated for 35 cycles: denaturation for 1 min at 94°C, annealing for 1 min and 35 s at 55°C and extension for 1 min at 72°C. The amplified sequence was digested by *Sfa*NI restriction enzyme (New England BioLabs, Beverly, Massachusetts, USA) at 37°C overnight. The digested products were electrophoresed in 2% agarose gel and visualized by ethidium bromide staining. The GG genotype corresponded to the presence of 140 and 58 bp fragments. The GC genotype corresponded to the presence of 198, 140, and 58 bp fragments. The CC genotype corresponded to a 198 bp fragment.

Demographic and clinical data between groups were compared by χ^2 test and by *t* test. Genotype and allele frequencies were compared by χ^2 test. Odds ratios were calculated with 95% CI. To estimate the association between genotype and OA, a logistic regression analysis was used (intercooled stata 6.0 (STATA) Statistics/Data Analysis STATA Corporation). Statistical significance was established at $P < 0.05$.

Results

There were no significant differences between groups in terms of age, sex and mean BMI. In the patient group, the mean age was 70.4 ± 7.9 years. In the control group, the mean age was 71.7 ± 10.8 years ($P = 0.3$). The male/female ratio was 30/45 in patients and 55/52 in controls ($P = 0.1$). In addition mean BMIs were not statistically different between groups, respectively, 28.05 ± 4.09 in patients with hip OA and 28.4 ± 2.82 in the control group ($P = 0.47$).

The distribution of IL-6 genotypes and alleles in cases and controls is shown in Table I. Genotypes were in Hardy–Weinberg equilibrium. In 75 patients with hip OA, the genotype distribution was 33 GG, 30 GC, 12 CC. Such distribution differed significantly from that observed in the 107 control subjects: 34 GG, 40 GC, 33 CC. The frequency of the CC genotype in controls was almost two times higher than in patients (30.8% vs 16.0%, $P = 0.02$). In contrast, the frequency of the GG genotype was higher in subjects

Table I
IL-6 genotype and allele distribution between groups

	OA ($n = 75$)	Controls ($n = 107$)	*P*
Genotypes			
G/G	33	34	0.09
G/C	30	40	0.7
C/C	12	33	0.022
Alleles			
G	96	108	
C	54	106	0.01

with OA than in controls, but this difference did not reach statistical significance (44.0% vs 31.8%, $P = 0.09$). The C allele was found in 49.5% of controls subjects and in 36.0% of patients, while the G allele was detected in 64.0% of patients with OA and in 50.5% of controls ($P = 0.01$).

A logistic regression analysis (Table II) showed that the CC genotype is independently associated with reduced risk of OA of the hip (odds ratio 0.4 [95% CI 0.1−0.9], $P = 0.04$).

Discussion

Inflammatory mechanisms play a crucial role in the pathogenesis and evolution of cartilage degradation. A large body of evidence suggests that lymphocytes, macrophages, and several forms of soluble inflammatory mediators are important in degenerative rheumatologic and orthopedic diseases. OA is characterized by degeneration of cartilage, which is expression of inflammatory reaction[11]. Recent studies have suggested that OA might be considered a chronic inflammatory disorder[12], and elevated levels of IL-1, tumor necrosis factor-α, IL-6 and other acute-phase proteins are found in patients with cartilage degradation[13].

Genes encoding for cytokines have been associated with susceptibility for joint OA. Recent studies indicate a role for the IL-1 gene cluster and the IL-1 receptor antagonist (IL-1RA) gene in the development of OA of the hip[4,5]. IL-6 gene is also supposed to be involved in the cartilage degradation process: in fact it is demonstrated that IL-6 expression is strongly induced in articular chondrocytes by IL-1 (see Ref.[14]). Importantly, the G allele of the −174 IL-6 gene polymorphism has been associated with an increased transcriptional response *in vitro* to stimuli, such as endotoxin or IL-1 (see Refs.[6,15]). In addition, Fishman *et al.*[6] have demonstrated that non-stimulated IL-6 concentrations are associated with the G allele in healthy individuals. Moreover, a recent study where healthy individuals homozygous for the respective alleles were exposed to a standardized inflammatory stimulus obtained by vaccination against *Salmonella typhii* has shown that the 174 G > C SNP in the promoter region of the IL-6 gene is functional *in vivo* with an increased inflammatory response associated with the G allele[16]. Our data suggest that IL-6 gene polymorphism could play a role in the process of the cartilage degradation. IL-6 in fact regulates production of many acute-phase proteins[17], promoting and maintaining the inflammatory phenotype. Because this interaction and amplification process would change on the basis of IL-6 levels, the clinical relevance of the IL-6 gene polymorphism is based on the fact that its gene variants influence levels and functional activity of the protein. In this sense our results show that the CC genotype of the −174 G/C polymorphism of IL-6 gene promoter determines a lower risk profile for osteoarthritic disease. We also suggest that the lack of association between the GG genotype and an increased risk of OA could be due to the limited number of patients involved in this study. This study has some potential limitations. The size of the study population is relatively small and our findings need to be confirmed in larger samples. The association between the IL-6 gene polymorphism and OA should be tested in different ethnic groups. This is a case−control study and a possible survival bias cannot be excluded for the group of patients with OA. Finally, we cannot exclude that the observed association depends on a gene in linkage disequilibrium with the IL-6 gene and further studies would be necessary to evaluate the real role of the −174 G/C polymorphism of IL-6 gene promoter in OA.

Table II
Risk factors of OA based on logistic regression analysis

	Odds ratio	CI (95%)	*P*
C/C genotype	0.4	0.1−0.9	0.04
G/C genotype	0.7	0.4−1.5	0.5
Sex M	0.69	0.3−1.2	0.2
BMI	0.98	0.9−1.08	0.79
Age	0.99	0.9−1.02	0.66

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